Two types of polyamine oxidases catalyzing the back-conversion pathway or terminal catabolism pathway co-exist in Oryza sativa

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（イネには逆変換反応型と末端分解型の２つのタイプのポリアミン酸化酵素が共存している）

分子応答制御分野

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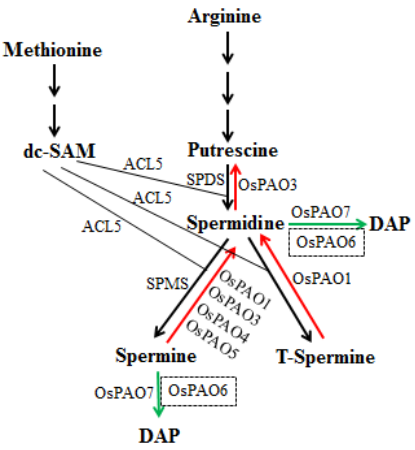
Major components of polyamines (PAs) in higher plants are diamine putrescine (Put), triamine spermidine (Spd), and tetraamines spermine (Spm) and thermospermine (T-Spm). Those amine compounds have fundamental roles in not only growth and development but also adaptive responses to various environmental stresses. The PA concentration is controlled by a dynamic balance between biosynthesis and catabolism. The latter process is governed by two enzymes, namely copper-contained amine oxidase and flavine adenine dinucleotide-associated polyamine oxidase (PAO). The best studied plant PAOs are maize PAO (ZmPAO) and two barley PAOs (HvPAO1 and HvPAO2). The analyses of reaction products revealed that those PAOs produce 4-aminobutanal from Spd and *N*-(3-aminopropyl)-4-aminobutanal from Spm, respectively, along with 1,3-diaminopropane (DAP) and H_2O_2. This type of catabolic pathway is named PA terminal catabolism (TC). On the other hand, mammalian PAOs convert Spm and Spd to Spd and Put, respectively, after the PA substrate is acetylated by Spm/Spd acetyltransferase. Mammals also have Spm oxidases (SMOs) which convert Spm to Spd without acetyl modification. Mammalian PAOs and SMOs are categorized into back conversion (BC)-type PAO.

The *Oryza sativa* genome contains seven PAO-encoded genes and they were termed *OsPAO1* to *OsPAO7*. One year ago, Ono et al (2012) characterized the expression of three *OsPAO*s, *OsPAO3, OsPAO4* and *OsPAO5*, and their genes' products. Their expression is rather abundant in entire growth stages. The recombinant proteins, OsPAO3, OsPAO4 and OsPAO5, catalyze BC-type reactions.

With the above background, in this study, I focus on two *OsPAO* genes, the one is *OsPAO1* and the other is *OsPAO7*. In rice plant, expression of *OsPAO1* seems to be quite low under physiological conditions, but is markedly induced by Spm or T-Spm treatment in a root-specific manner, suggesting that it is involved in tetraamine catabolism. In accord with this speculation, the recombinant OsPAO1 prefers T-Spm as a substrate at pH 6.0 and Spm at pH 8.5 and, in both cases, back-converts these tetraamines to Spd but not further to Put. OsPAO1 localizes to the cytoplasm of onion epidermal cells. The enzymatic behavior and subcellular localization of OsPAO1 are quite similar to those of AtPAO5 of *Arabidopsis thaliana*. Furthermore, the *Atpao5* mutant showed growth arrest of its aerial parts, but not roots, when

grown on MS agar medium containing low doses (5 or 10 μM) of T-Spm. This effect was specific to T-Spm because WT and *Atpao5* mutants showing almost comparable growth on 1 mM Put-, 1 mM Spd- or 300 μM Spm-contained media. Introduction of *OsPAO1* directed by a constitutively expressed promoter into the *Atpao5* mutant recovered the growth arrest of the host plant in the presence of low doses T-Spm. OsPAO3 did not have such activity. Taken all, I propose that OsPAO1 is an ortholog of Arabidopsis AtPAO5 and functions as a T-Spm oxidase in rice. Next *OsPAO7* and its product enzyme are studied. *In silico* data suggest that *OsPAO7* is specifically expressed in anther organ. In fact, I was able to isolate its cDNA from flower organ. OsPAO7 is localized in a peripheral layer of plant cells with the aid of its predicted signal peptide and transmembrane region, suggesting that OsPAO7 is an apoplastic enzyme. As expected from the high identity of OsPAO7 to ZmPAO, HvPAO1 and HvPAO2, the recombinant OsPAO7 produces DAP from both Spm and Spd in a time-dependent manner. It indicates that OsPAO7 is a first TC-type enzyme in *O. sativa*. Furthermore, *OsPAO7* is specifically expressed in anther organ with an expressional peak at the bicellular pollen stage.



In *Oryza sativa*, two-types of PAOs co-exist. Namely, of seven OsPAOs, OsPAO1 (this study), OsPAO3, OsPAO4 and OsPAO5 catalyze BC-type reactions whereas OsPAO7 (this study) catalyzes a TC-type reaction (see the left figure). While almost all the OsPAO proteins are consisting of 474-500 amino acids, OsPAO2 is a 351-amino acid-protein and lacks the N-terminal portion that harbors the part of catalytically essential residues. Therefore, I assumed that OsPAO2 might lack PAO enzyme activity. Meanwhile, OsPAO6 is expected to have TC-type PAO enzyme activity because it shows high identity to OsPAO7 and other TC-type PAO members in the entire protein region. More distinct function of each OsPAO member will be investigated in the future works.

Publication list

- **Taibo Liu** et al. (2014) Plant Cell Reports in press, doi:10.1007/s00299-013-1518-y.
- G.H.M. Sagor†, **Taibo Liu**† (2013) Plant Cell Reports 32:1477–1488.
  (†contributed equally to this work)
- **Taibo Liu** et al. Journal of Experimental Botany (under review)